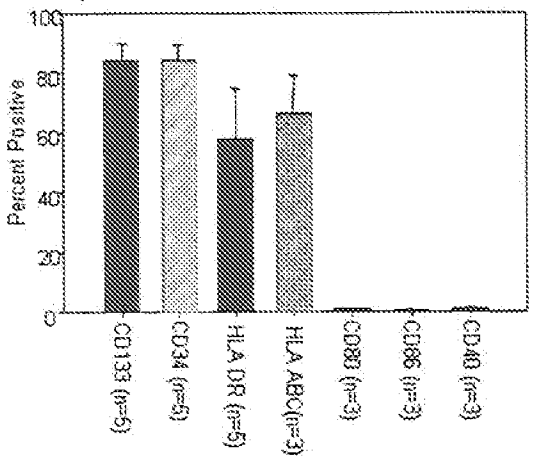
ATTACHMENT B

Fig 1



UCB CD133+ cells express HLA class I and II antigens and do not express co-stimulatory antigens (Fig 1). UCB CD133$^+$ HSC co-express CD34 and express class I (HLA-ABC) and class II (HLA-DR) but **lack expression of co-stimulatory antigens** including **CD40, CD80 and CD86**. Co-stimulatory antigens are required for full T-cell activation and their absence on antigen presenting cells (APC) leads to T-cell anergy, due to absent binding and co-stimulation of T-cells via CD28 engagement [9]. Nevertheless, whether the immunogenicity of a CD133$^+$ allogeneic therapeutic cellular product is advantageous to augment angiogenesis by recipient endothelial cells *in situ* or potentially deleterious in dampening angiogenesis response or responsible for worsening vascular ischemia via allogeneic inflammatory responses is unknown; and warrants administration of low cell doses of CD133 therapeutic cellular product in a phase I dose escalation approach.

UCB CD133$^+$ HSC and human MSC synergistically promote vasculogenesis in response to ischemia. Preliminary studies conducted in Dr. Laughlin's laboratory at Case Western Reserve University tested human bone marrow (BM)-derived mesenchymal stem cells (MSC) injected alone and in combination with UCB-derived CD133$^+$ HSC. Significantly enhanced blood flow and histologic evidence of angiogenesis was noted in this NOD.SCID *in vivo* hindlimb femoral ligation model (Fig 2). Femoral ligation and resection was performed and study animals were randomized to one of three treatment groups. Group 1, control, was treated with injection of media (0.02 ml). Group 2 animals received third passage human MSC ($1x10^6$ in 0.02 ml). Group 3 animals received both UCB CD133$^+$ HSC and MSC at an equivalent total cell dose ($0.5x10^6$ + $0.5x10^6$ in 0.02 ml; total combined human cell dose $1x10^6$ in 0.04 ml). The animals were survived for 6 weeks. There were significant differences in the Doppler blood flow ratio measured at day 42 among the three conditions. Pair-wise comparison revealed significantly higher blood flow measured in animals injected with both MSC and UCB 133$^+$ HSC compared with those animals treated with MSC alone ($p<0.05$). Taken together, these preliminary reports point to potential synergy between endogenous endothelial, inflammatory, and stromal cells and administered hematopoietic stem and mesenchymal cells in mediating murine angiogenesis responses to ischemic vessel injury.

Fig 2